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ASCCIACION CIENCIA E INVESTIGACION
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POTENTE ACCION LIPOTROPICA...

- INFILTRACION GRASA DEL HIGADO
- CIRROSIS HEPATICA
- HEPATITIS

Su sabor agradable asegura la continuidad del tratamiento

COLINA
INOSITOL
ACIDO FOLICO
VITAMINA B_{12}

HICOSITON

Dosis: 1 cucharada tres veces al día
Presentación: Frasco 120 y 240 cm^3

SQUIBB

"HICOSITON" ES MARCA REGISTRADA

ACROMICINA

HCl DE TETRACICLINA CRISTALINA

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INTRAMUSCULAR

ENVASE: FRASCO AMPOLLA POR 100 mg.

...el antibiótico de espectro verdaderamente amplio

PASA A PRIMER PLANO CUANDO:

EL MEDICO DESEA ESTRICTO CONTROL
EL ENFERMO NO PUEDA INGERIR
EL PEDIATRA NO DESEE UTILIZAR LA VIA ORAL (TOXICOSIS, DESHIDRATACION)
EL CLINICO NO DESEE TERAPIA ORAL
EL CIRUJANO PRECISE LA VIA INTRAMUSCULAR
EL OTORALINGOLOGO TROPIECE CON DISFAGIA
EL OBSTETRA DEBA ENFRENTAR ESTADOS NAUSEOSOS

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LAS PRUEBAS CLINICAS CERTIFICAN SU EFICACIA

Announcement

Acta Physiologica Latinoamericana, in an effort to provide better reading material and always careful to comply with the highest standards, has started a new section "Reviews". This section will appear twice a year and in alternate numbers of the journal. The aim will be to provide an opportunity to distinguished researchers in different fields of the physiological sciences to present their views in comprehensive form. A mere review of the literature is discouraged because there are excellent publications which provide ample reading material and abundant references. In fact, the scope of this section is wide enough to allow any author a detailed account of work done in the past with special reference to the general implications which such work would provide.

Collaborations are accepted by invitation only and papers submitted without a formal request will be discarded. Manuscripts should be typewritten in triple space and with neat diagrams and illustrations. There is no limitation of space of figures, but if figures are excessive with regard to the length of the paper, the Editorial Office will discuss with the author the possibility of eliminating a few of them. Each collaboration must comply with the following rules: one original and one carbon copy should be sent with unmounted illustrations in a separate envelope but inside the same cover. The author must indicate in a separate sheet the source of illustrations already published in order to secure permission from the editors to reproduce them. Also, all manuscripts should be accompanied by a reprint order in which the authors will indicate the exact number of reprints desired.

Papers should be sent to Dr. Carlos Eyzaguirre, Laboratorio de Neurofisiología, Universidad Católica de Chile, Casilla 114-D, Santiago, Chile.

THE SYNAPTIC TRANSMISSION OF SYMPATHETIC GANGLIA DURING WALLERIAN DEGENERATION. EFFECT OF LENGTH OF DEGENERATING NERVE FIBERS *

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THE ONSET of neuromuscular alterations during Wallerian degeneration (W. d.) of the motor nerve is closely related to the length of the axon. Muscular elements nearest to the nerve section present the earliest changes, while more distal regions are affected at a later period (Eyzaguirre, Espíldora and Luco, 1952). Furthermore, fibrillation and hypersensitivity to acetylcholine, which are known to appear a few days after nerve severance, follow an analogous time course (Luco and Eyzaguirre, 1955). Similarly, in a recent paper Vial (1955) has demonstrated that the length of an axon undergoing degeneration influences the appearance of the anatomical features of degeneration in the motor end-plates.

It seemed interesting to study the effect of the length of degenerating presynaptic nerve fibers on a neuro-neural junction such as the synapse of the superior cervical ganglion of the cat.

METHODS

Fifty five unselected cats were employed. In order to induce W. d., the preganglionic fibers were either crushed or cut under ether anesthesia. On one side the nerve was severed 2 cm centrally to the ganglion and on the other side at 7 cm. The former will be designated as "short nerve preparation" and the latter as "long nerve preparation". In all cases the cervical sympathetic was carefully studied and animals presenting a middle cervical ganglion were discarded. Sodium Pentobarbital (Nembutal Abbot, 0.6 cc per kilogram) was employed for anesthesia in the acute experiment. The superior portion of the trachea, the pharynx, the upper part of the esophagus and the pre-vertebral muscles were removed in order to form a pool which was filled with mineral

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oil for stimulation and recording. The temperature was maintained at about 36° C. The stimulating leads were placed on the preganglionic fibers near the ganglion in an intact portion of the nerve. The recording electrodes (platinum wire) were placed on the postganglionic fibers: one near the superior pole of the ganglion and the other one 2 to 3 mm distally on a previously crushed segment. The preganglionic nerve of the long nerve preparation was also crushed above the stimulating elec-

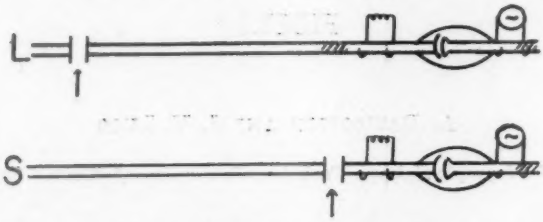

FIG. 1. — *Schematic diagram of the preparation.* L, *long nerve preparation.* S, *short nerve preparation. The arrows indicate the site of section of preganglionic fibers. Shaded areas show crushed regions.*

trodes. Fig. 1 shows a diagram of both preparations. A Grass model S4A stimulator was used. The duration of the pulses ranged from 0.01 to 0.05 msec. Preamplifiers (Grass P 4) and a two-beam cathode ray oscillograph were employed to record electrical responses.

RESULTS

A. *Responses to single stimuli.* — Coppée and Bacq (1938) observed that during W. d. synaptic transmission failed before the preganglionic fibers ceased to conduct. Our results agree, as a whole, with this observation and contribute some additional information.

Alterations of the synaptic mechanism were observed from 24 hours after nerve section and could be followed up to 70 or 90 hours when synaptic transmission failed completely. Fig. 2 shows a typical example. The voltages of the recorded postsynaptic potentials decrease during the temporal progression of W. d. The response becomes flat, the phases are no longer present and the positive after potential decreases (B to G). In an advanced stage of degeneration, when the propagated alterations are very small, a negative potential appears with all the characteristics of a synaptic potential (G). This observation is similar to that reported by Eyzaguirre, Espíldora and Luco (1952) in a neuromuscular preparation during W. d.

A comparison between the behaviour of the long and short nerve preparations seems of interest. The response registered at E is very similar to that registered at C, i. e., a short nerve preparation denervated for 24 hours behaves like a long nerve preparation of 48 hours. Similarly as another instance of the same phenomenon F is comparable to D. Responses like G have been observed in a long nerve preparation of 80 to 90 hours of degeneration.

In other words, both preparations show the same graded alterations, but their temporal course is different. Changes in the response of the short nerve preparation precede those of the long one.

B. *Post-tetanic effect.* — The augmented response observed after tetanic stimulation has been studied in different synaptic structures. (Rosenblueth and Morison, 1937; Brown and von Euler, 1938; Lloyd,

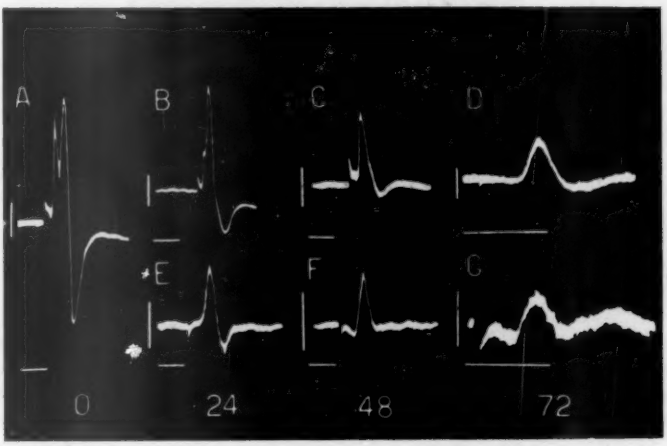

FIG. 2. — *Postsynaptic responses to single presynaptic stimulation.* A, *normal preparation.* B, C, *and* D, *long nerve preparation.* E, F, *and* G, *short nerve preparation. Hours after nerve section appear below each column. Time: 20 msec. Calibration in microvolts:* A, *50;* B, *and* E, *100;* C *and* F, *50;* D, *40;* G, *20.*

1949; Eccles and Rall, 1951) and it was considered interesting to record the post-tetanic effect during W. d., because it essentially depends on the properties of the presynaptic ending (Larrabee and Bronk, 1947; Liley and North, 1953).

The experiments were performed using single maximal shocks delivered regularly every few seconds. For repetitive maximal stimulation, trains were applied at a frequency of 5 to 30 stimuli per second and lasting from 1 to 30 seconds. As in the previous series the degenerative process was observed from 24 up to 90 hours after nerve section.

During the early stages of degeneration post-tetanic potentiation was less pronounced than in the normal preparation. At a later stage the post-tetanic potentiation was replaced by a depression of the single response. This depression is illustrated in Fig. 3. Later on, in a well advanced stage of W. d. the post-tetanic potentiation reappeared, although its intensity was less pronounced (Fig. 4).

It is well known that the positive after potential is depressed after repetitive stimulation of a normal ganglion (Rosenblueth and Simeone, 1938). During W. d. this effect is twofold: a) during the early stages

of degeneration the positive after potential behaves as in the normal preparation, i. e., is depressed after a tetanus (Fig. 3); b) in a more advanced stage of degeneration this effect is reversed and the after-positivity increases after tetanic stimulation (Fig. 4).

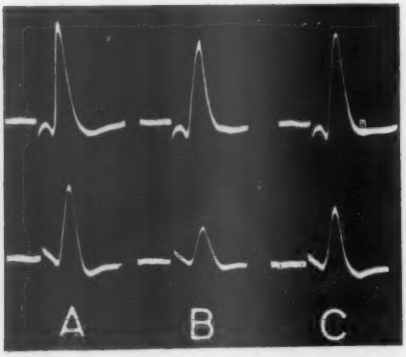

FIG. 3. — *Post tetanic effect at 40 hours after nerve section. Maximal single stimulation. Upper row, long nerve preparation. Lower row, short nerve preparation.* A: *Before tetanus.* B: *3 sec after tetanic stimulation at 30 per sec for 5 sec.* C: *3 sec after* B.

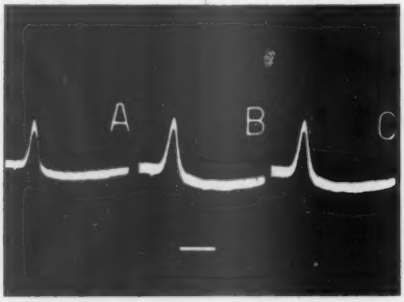

FIG. 4. — *Post tetanic effect at 74 hours after nerve section. Maximal stimulation.* A: *Before tetanus.* B: *2 sec after tetanic stimulation at 30 per sec for 5 sec.* C: *20 sec after* B. *Time calibration 20 msec.*

Fig. 3 illustrates that during a relatively early stage the response of the long nerve preparation remains practically unchanged after a short tetanus while the short nerve preparation shows a clear post-tetanic depression. This different behaviour of the two preparations is better illustrated in Fig. 5. Tetanic stimulation at 30 per second

during 2 second duration may be followed by a potentiation in the long nerve preparation and by a depression in the short nerve one. Furthermore, if the tetanus is made longer the long nerve side shows a small decrease of the response while on the short nerve side this depression is far more marked.

Some fatigued preparations after 70 or more hours of degeneration show a very interesting post-tetanic response. Tetani applied at

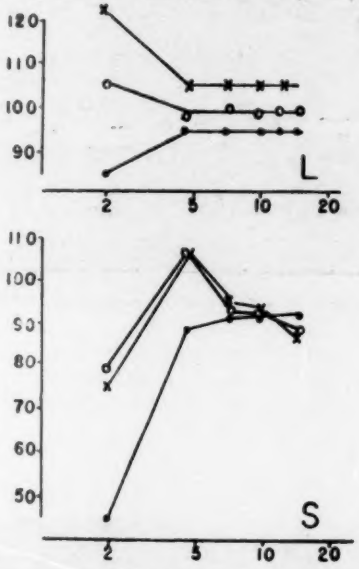

FIG. 5. — *Effect of tetanus duration on the post-tetanic reaction 50 hours after nerve section.* L, *long nerve preparation.* S, *short nerve preparation. Ordinates, amplitude of the action potential expressed in percentage (control response equal 100 %). Abscissae, time in sec after tetanus at 30 per sec. Crosses, 2 sec duration. Open circles, 3 sec duration. Full circles, 4 sec duration.*

frequencies ranging between 10 and 30 per second and lasting for periods from 5 to 20 seconds produce a depression of the negative action potential and the persistance of the positive potential. As the preparation recovers the negative action potential reappears while the positive deflection becomes less obvious (Fig. 6).

C. *Synaptic fatigue.* — In this series 17 experiments were performed 24 to 90 hours after nerve section. The frequency of stimulation ranged from 5 to 60 per second. Special care was taken to ensure maximal stimulation and similar experimental conditions for the long and short nerve preparations. Fatigue was measured by the amplitude of the negative propagated potential.

As it would be expected. synaptic fatigability of a sympathetic ganglion under W. d. of the preganglionic fibers increases during the progress of the degenerative process. Fig. 7 shows the fatigability of

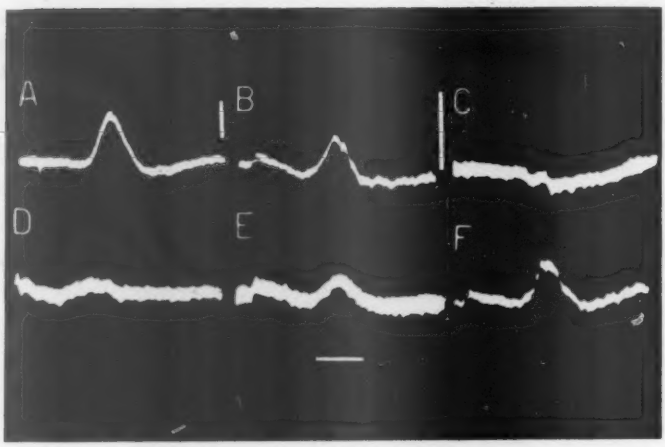

FIG. 6. — *Post tetanic effect in a fatigued preparation of 72 hours of W. d. Maximal stimulation.* A: *Control.* B: *During fatique and before the tetanus.* C: *2 sec after tetanic stimulation at 30 per sec applied during 10 sec.* D: *2 sec after* C. E: *30 sec after* D. F: *6 m after* E. *Calibration for* A *(in* A*): 40 microvolts. Calibration for* B *to* F *(in* B*): 40 microvolts. Time equal 10 msec.*

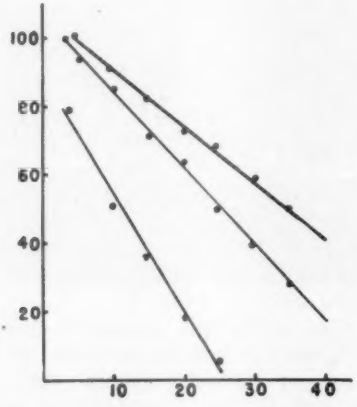

FIG. 7. — *Fatigability at different time of degeneration. Maximal stimulation at 60 per second. Upper curve: normal preparation. Middle curve: 24 hours after section. Lower curve: 50 hours after nerve section. Ordinates: amplitude of the negative potential expressed in percentage of the response to the first stimulus. Abscissae:number of stimuli.*

a normal preparation stimulated at a frequency of 60 per second and the fatigability of two ganglia undergoing degeneration, 24 and 50 hours after nerve severance.

When fatigability of the long nerve preparation is compared with that of the short nerve the same general conclusions as described in the above series are reached. Fig. 8 illustrates this phenomenon. The

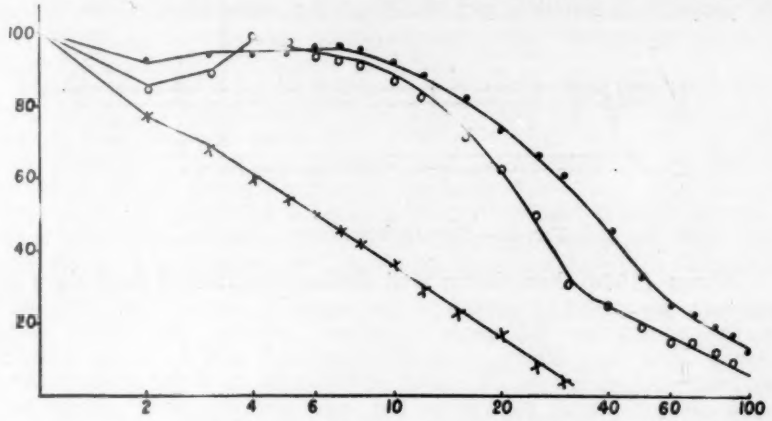

FIG. 8. — *Fatigability of the normal (full circles), long (open circles) and short (crosses) nerve preparations. Maximal stimulation at 60 per sec. Ordinates and abscissae as fig. 7.*

preparation, studied 24 hours after nerve section, was stimulated at 60 per second. As it has been described in a normal preparation (Rosenblueth and Simeone, 1938) the second stimulus produces a response slightly smaller than the previous one; afterwards it increases, to decrease later on because of the presence of fatigue. The long nerve preparation behaves like the normal ganglion, the main difference being that its fatigability is slightly more pronounced. On the other hand, the short nerve preparation is more easily fatigued.

DISCUSSION

A. *Degenerating presynaptic fibers.* — Before discussing the synaptic function of a nerve it seems necessary to analyze the conduction of nerve impulses in a degenerating nerve axon. Rosenblueth and del Pozo (1943) demonstrated that alterations in the conduction of nerve impulse due to W. d. follows a centrifugal course. They were able to observe in a nerve severed by two sections that the peripheral cut does not alter the gradient produced by the higher section, i. e., a single gradient was present for the nerve as a whole.

Causey and Stratman (1954) have confirmed this centrifugal course. Luco and Bozzo (unpublished data) also observed that each nerve locus stops conducting at a given moment which does not depend

on its distance to the site of section. In cats both peroneal nerves were cut simultaneously, one in the higher portion of the thigh and the other 7 to 8 cm further down. The conduction of the nerve impulse was studied 24 hours after nerve section up to 80 or 100 hours when the nerve stops conducting. Homologous peripheral nerve segments were studied (see diagram of Fig. 9). The nerves of both sides stop conducting propagated impulses practically at the same time.

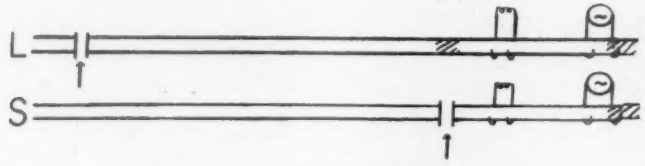

FIG. 9. — *For explanation see Fig. 1.*

Gibson (1940) has shown that during the first 2 or 3 days after severance preganglionic nerve of the superior cervical ganglion of the cat the conduction of nerve impulses remains practically normal. After 4 days these fibers were little excitable and could be stimulated only near the ganglion. It seems justificable to infer that under the present experimental conditions the long and the short nerve preparation behave similarly with regards to conduction of nerve impulses and are both comparable to the normal preparation.

B. *Synaptic transmission.* — The results reported here show that there is a graded failure of synaptic transmission at a neuro-neural synapse during the development of W. d. On the other hand, this failure cannot be correlated with alterations in the conduction of nerve impulses by the axon as it has been discussed above. The mechanism of this failure has to be found in some other property of the presynaptic element. Two possibilities seem more probable: a) alteration of nerve impulse conduction at the fine terminal arborization and b) biochemical modifications of the acetylcholine metabolism.

With regard to the first possibility there are not many relevant data in the literature and it seems doubtful that knowledge of the physiological behaviour of the fine terminals can be obtained as an extrapolation of axonic data. Laporte and Lorente de Nó (1952) consider the sensitivity of the nerve endings to curare. Liley and North (1953) suggest that the post-tetanic potentiation depends on an increased electrical response of the terminals. In view of the fact that the nerve terminals have not been extensively studied during W. d. this problem remanis still open.

The second posibility has been better studied and the literature offers more data, allowing the discussion to be more complete. It is well known that acetylcholine content of the ganglion decreases during degeneration and it is only 20 % of the normal value 70 hours after nerve section (MacIntosh, 1938). Acetylcholine synthesis is also diminished, which is interpreted as being due to a defficiency of cholinacetylase in the nerve endings (Feldberg, 1943; Banister and Scrase,

1950). The concentration of local cholinesterase is also decreased (Croxatto, Huidobro and Luco, 1940; Sawyer and Hollinshead, 1945). All these observations are showing that during W. d. abnormal metabolic conditions alter the chemical transmitter effect.

From the above considerations it may follow that the responses observed after single stimuli can be due to a decrease of active postsynaptic elements because of insufficient amount of acetylcholine liberated. In an advanced stage of degeneration the transmitter would be able to build up only a synaptic response.

With regard to the fatigability of a ganglion with the presynaptic fibers undergoing degeneration, it is well known that prolonged stimulation of a cholinergic nerve at a relatively high frequency produces synaptic fatigue. This phenomenon has been explained as follows: the rate at which the trasmitter is synthetized is lower than the rate of liberation, therefore an increasing deficit of acetylcholine would be present (see Rosenblueth, 1950). During degeneration of the presynaptic fibers this phenomenon should be more marked and fatigability should appear more intense.

The post-tetanic facilitation is a conditioning effect of the presynaptic element (Larrabee and Bronk, 1947) and, in accordance with the explanation of Liley and North (1953), an increase in the negative potential of the nerve endings would liberate a larger amount of acetylcholine which in turn would induce a larger response after the tetani. Under our experimental conditions, the diminution or absence of post-tetanic facilitation may be explained as a consequence of a disturbed acetylcholine metabolisme during W. d.

The post-tetanic depression observed in a relatively advanced degeneration is probably due to the great fatigability during W. d. In order to explain the appearance of a small post-tetanic potentiation during well advanced degeneration (see results and Fig. 4) it seems necessary to take into account the hypersensitivity of denervated structures (see Cannon and Rosenblueth, 1949). This increment is present when sensitization is frankly appreciable and in spite of the probable diminution of liberated acetylcholine, it might give a greater response after tetani. This observation is in some way confirmatory of the results reported by Simeone, Cannon and Rosenblueth (1938). They observed that a partially denervated sympathetic ganglion presents a longer post-tetanic effect when the normal preganglionic fibers were stimulated.

It is interesting to discuss the probable meaning of the positive potential illustrated in Fig. 6. Laporte and Lorente de Nó (1950) described a positive potential in the curarized sympathetic ganglion of the turtle. This potential is also potentiated by tetanic stimulation. They suggest that curare does not block certain types of fibers which normally produce a positive response and would have an inhibitory effect. Eccles (1952) has confirmed this observation, but she explains this effect as due to the possibility that curare would change the depolarizing effect of acetylcholine into a hyperpolarizing one. Fatt (1954) suggests another explanation: positivity would result from acetylcholine acting in a region of the postsynaptic membrane different than the one that giving a negative response.

The positive response reported in this paper appeared without the administration of curare, but in a fatigued ganglion during a well advanced stage of degeneration. There are many possible inferences but the following seems to be more probable: Curare, fatigue and W. d. are not indispensable factors of the appearance of this positive potential. It might be that normally it is not recorded, being obscured by the much larger negative responses.

The meaning of this positive potential is still uncertain although it is suggestive that it might have an inhibittory action, which is in line with the observation of Lorente de Nó and Laporte (1950). Although positive potentials have been identified as concomitant with inhibition (Brock, Coombs and Eccles, 1952), it is true that inhibition may be present without any detectable potential (Fatt and Katz, 1953) or with a frank depolarization (Kuffler and Eyzaguirre, 1955).

C. *Effect of length of degenerating nerve fibers.* — The main purpose of the present study was to test in a neuro-neural synapse the effect of length of degenerating presynaptic nerve fibers. The study of this effect was done by comparing the long nerve preparation with the short nerve one in the same animal and under similar experimental conditions. Our results show a direct correlation between the length of the severed portion of an axon and the time of onset of synaptic alterations. The three tests used here namely, response to single stimuli, fatigability and post-tetanic effect, demonstrate that if the isolated portion of the presynaptic fibers is short, all alterations of synaptic transmission appear earlier than when a greater length of nerve is left to degenerate.

No matter which is the mechanism of synaptic disturbances during W. d.: metabolic alterations of acetylcholine or other abnormal behaviour of the nerve terminals, our results reveal that the axonic portions located at some distance from the junction seem to have an influence on the synapse. These portions delay the appearance of synaptic disturbances due to W. d. These results, confirm as a whole the conclusion reached by Eyzaguirre, Espíldora and Luco (1952), by Luco and Eyzaguirre (1955) and by Vial (1955).

Summary

The onset of synaptic alterations during Wallerian degeneration of the presynaptic fibers was studied in the superior cervical ganglion of the cat.

On one side the preganglionic fibers were cut near the ganglion and on the other the nerve was severed at a more distal location. Both preparations were tested after single stimuli, fatigue and to the post-tetanic effect.

The onset of synaptic modifications appeared earlier in ganglia denervated by a close cut than in ganglia denervated by a distal section.

It is concluded that the development of synaptic disturbances by Wallerian degeneration is conditioned by the length of the degenerating neuron.

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ANEMIA HEMOLÍTICA PROVOCADA POR LA ESPLENECTOMÍA EN LA RATA BLANCA PORTADORA DE BARTONELLAS

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LA ANEMIA infecciosa de las ratas es una afección conocida desde hace tiempo y de la que se han ocupado parcialmente varios autores. Ya en 1921 Mayer [1] describió como su agente causal un microorganismo que llamó "Bartonella muris" y posteriormente Friedberg [2], Koh [3], Perla y Marmorston-Gottesmann [4], Bacigalupo [8, 9] y Lauda [10] entre otros, describieron distintos aspectos de la enfermedad, e intentaron explicar el mecanismo de la protección esplénica frente a la infección, llegando hasta atribuirla a una secreción hormonal. Estos trabajos, sin embargo, no han sido continuados hasta obtener conclusiones definitivas.

Consideramos que el síndrome de anemia hemolítica aguda por bartonelosis proporciona un excelente instrumento de trabajo para el estudio de algunos aspectos de la fisiología esplénica, así como de la patología de las anemias hemolíticas agudas.

El objeto de este trabajo es describir en detalle el cuadro de la enfermedad y estudiar algunos posibles mecanismos de la protección esplénica.

MATERIAL Y MÉTODOS

Se usaron en los distintos experimentos alrededor de 200 ratas blancas de nuestro criadero, adultas de ambos sexos y de pesos variables entre 150 y 300 g.

Las esplenectomías se realizaron bajo anestesia etérea, por vía lumbar, sin ligar el pedículo esplénico porque la pequeña hemorragia operatoria cohibe espontáneamente. Igualmente en las esplenectomías parciales no se usó sutura ni ligadura de ninguna clase excepto para la piel.

Los exámenes hematológicos se practicaron con sangre de la cola, con las técnicas habituales. Los extendidos fueron coloreados con May Grünwald-Giemsa y cuando se necesitó la colaboración supravital, con azul brillante de Cresil.

Para la esterilización de los animales se usaron distintas sales de

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arsénico; y en particular Sulfarsenol 1 mg/100 g de peso disuelto en agua destilada, por vía intraperitoneal.

El homogeneizado de bazo se preparó moliendo en un mortero bazos recién extraídos, con solución fisiológica. Luego se filtró con papel de filtro y sin esterilizar se inyectó por vía intraperitoneal.

Para estudiar la producción de anticuerpos se inyectaron ratas con glóbulos rojos de cobayo (1 dosis de 0.25 cc por vía subcutánea) y 7 a 15 días después se obtuvo el suero de inactivado 30′ a 56° se puso en contacto con el antígeno en portaobjeto y en tubo a 37°.

RESULTADOS

I) *Anemia hemolítica*

a) *Cuadro clínico.* — Durante los tres primeros días siguientes a la operación no se advierten modificaciones fundamentales del cuadro clínico excepto una pérdida de peso en las primeras 24 h atribuíbles al traumatismo y a la pequeña hemorragia operatoria. Hacia el 3º ó 4º día se instala el cuadro de la enfermedad, que se caracteriza por: palidez, disnea, hirsutismo, hipotermia, astenia y pérdida del tono muscular, disminución del apetito y del peso, y emisión de orina frecuentemente hematúrica. Los síntomas se acentúan con el tiempo y entre el 4º y el 7º día los animales mueren o, en un pequeño porcentaje, se sobreponen a la enfermedad y comienzan a recuperarse. La observación de estos animales durante largo plazo permite constatar recaídas ocasionales, particularmente en momentos críticos: enfermedad, preñez, etc., cualquiera de las cuales puede provocar la muerte, sin que aparentemente se haya desarrollado inmunidad desde que los distintos brotes agudos son de intensidad sensiblemente igual.

b) *Cuadro hemático.* — Glóbulos rojos: Coincidiendo con el cuadro clínico, durante los tres primeros días no se observa sino un descenso pequeño de la cifra de hematíes explicable por la hemorragia operatoria y la eliminación del reservorio esplénico, rápidamente compensadas. El 4º día, sin embargo, se observa un brusco descenso a menos de la mitad, y el 5º día las cifras de rojos alcanzan sus valores más bajos, entre 1 y 3 millones, o aún menos en algunos casos.

En los extendidos de sangre periférica se observa un cuadro intensamente regenerativo con marcada anisocitosis, poiquilocitosis, policromatofilia y eritroblastosis hasta de 50 a 100 mil elementos por milímetro cúbico.

La coloración supravital muestra que casi la totalidad de los glóbulos rojos en este momento son reticulocitos.

En los extendidos coloreados con May Grünwald-Giemsa pueden verse las bartonellas en el interior de los hematíes ya aisladas, ya agrupadas hasta llenar por completo algunos glóbulos. Su morfología variada ha sido estudiada en fresco por Reese y colaboradores con el microscopio de contraste de fase ([5]).

Glóbulos blancos: Al mismo tiempo que los rojos disminuyen se observa una brusca elevación de los glóbulos blancos, que alcanzan cifras hasta de 70 u 80 mil por milímetro cúbico según las técnicas corrientes.

La fórmula blanca, habitualmente con predominio linfocitario en la rata, se invierte mostrando una intensa neutrofilia de 80-90 % con presencia de elementos muy jóvenes, de protoplasma débilmente basófilo y núcleo poco lobulado. Como consecuencia de la leucocitosis y dada la inversión de la fórmula, la cifra de neutrófilos que normalmente oscila entre 3 y 5 mil elementos por milímetro cúbico alcanza valores entre 30 y 50 mil.

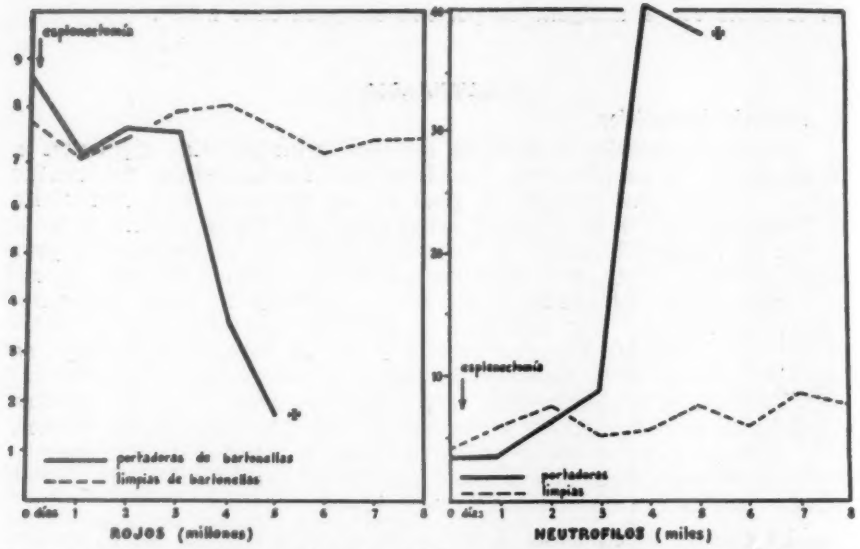

FIG. 1. — *Curvas de glóbulos rojos y neutrófilos en ratas esplenectomizadas:* 1) *portadoras de bartonellas, y* 2) *testigos tratadas con arsénico. 8 animales por grupo.*

c) *Resistencia globular.* — La resistencia de los hematíes a las soluciones salinas hipotónicas está muy disminuída comenzando la hemólisis en soluciones de ClNa al 6 por mil, mientras que la de los normales comienza en soluciones al 4-4.6 por mil. El final de la hemólisis es muy variable dependiendo del momento del proceso en que se toma la muestra.

d) *Autopsia.* — El examen macroscópico muestra una franca coloración amarillenta de las vísceras y las mucosas. El timo está atrófico y las suprarrenales hipertróficas. El hígado es pálido y en muchos casos muestra signos groseros de sobrecarga grasa. No se observan lesiones macroscópicas en pulmones, corazón, riñones ni en las demás glándulas endócrinas.

e) *Estudio histológico.* — Hígado: Presenta las mayores alteraciones: las células hepáticas están en su mayoría llenas de vacuolas de grasa de distinto tamaño. Las células de Kupffer se encuentran abarrotadas de restos de hematíes. Se observa además un infiltrado más o menos intenso de polinucleares, algunas veces difuso y otras circuns-

cripto en verdaderos microabscesos, los más grandes visibles a simple vista.

Riñones: Las células de los túbulos proximales se encuentran llenas de granulaciones eosinófilas y algunas vacuolas de degeneración grasa. Hay cilindros albuminoides en el interior de los túbulos. No se observa nefrosis del nefrón inferior.

Suprarrenales: Presentan hipertrofia de la zona reticular y signos de agotamiento en la fascicular.

f) *Morbimortalidad.* — De 170 animales adultos esplenectomizados ninguno dejó de presentar uno o más de los síntomas clínicos o hematológicos de la anemia hemolítica, por lo que consideramos la morbilidad como del 100 % entre nuestras ratas.

En cambio, no todos van a la muerte. De 170 esplenectomizados, 135 (79.4 %) murieron, la gran mayoría entre 4 y 10 días después de la operación, y 35 (20.6 %) después de anemizarse con intensidad variable comenzaron a recuperarse mejorando sus síntomas clínicos y normalizándose el peso alrededor de los 15 días.

Las bartonellas permanecieron visibles en los frotis de sangre periférica sólo alrededor de 5 días desde su aparición, o sean unos 8 ó 9 luego de la esplenectomía.

g) *Terapéutica.* — Se sabe que pueden esterilizarse los animales infectados usando sales de As [1]. Ha sido descripto además que con la inyección de dosis subdiabetógenas de aloxano [6] puede impedirse la diseminación de los gérmenes y se supone aunque no hay prueba definitiva que también de este modo se logra la desaparición total de las bartonellas.

Nosotros empleamos diferentes preparados comerciales de As, y en particular, Sulfarsenol, todos útiles, con la condición de no estar oxidados. La dosis es baja, bastando una única inyección intraperitoneal de 1 mg de Sulfarsenol por 100 g de rata, diluído en H_2O o solución fisiológica.

Esta terapéutica es eficaz, instituida antes de la esplenectomía o durante las primeras 48 h de la operación, o sea antes de la diseminación bacteriana. Una vez comenzada la hemólisis masiva, dosis aún 20 veces superiores han sido ineficaces.

h) *Transmisión.* — Ha sido atribuida a un parásito, probablemente el piojo de las ratas. Sin descartar otros medios posibles, hemos podido lograr la reinfección de ratas previamente esterilizadas parasitándolas con piojos provenientes de ratas comunes del criadero no esplenectomizadas, por lo que creemos que es la vía más común de transmisión en nuestros animales.

El estudio de otros medios posibles de transmisión: agua, comida, orina y materias fecales no ha permtido obtener conclusiones definitivas en uno u otro sentido.

I1) *Ausencia de hipertrofia compensadora de bazo*

Perla y Marmorston-Gottesmann lograron impedir el desarrollo de la infección en portadores dejando en su sitio una cuarta parte del bazo con buena irrigación.

Con el objeto de comprobar si frente al estímulo que significa la infección latente esa masa de bazo podía hipertrofiarse, se practicó a

10 animales una esplenectomía subtotal dejando aproximadamente 1/4 a 1/5 del órgano con buena irrigación, y se pesó el fragmento extirpado y 20 días después de la primera operación se extirpó el trozo restante a los 8 que sobrevivieron. Se sumaron los pesos de los dos fragmentos y se los comparó con los pesos de bazos normales. En ese lapso no

TABLA I

Pesos % de bazo

	n	$\bar{x} \pm$ e. s.	
Testigos	21	551.38 ± 41.6	F = 2.51 (p > 0.05)
Esplenectomizados en 2 tiempos	8	550.00 ± 21.5	
Esplenectomizados en 4 tiempos	12	668.33 ± 36.2	F = 4.78 (p < 0.05)

pudo apreciarse hipertrofia de ninguna clase.

Posteriormente se practicó la esplenectomía en 4 tiempos extrayéndose en el primero alrededor de 4/5 del órgano y en los siguientes la mitad del fragmento restante hasta el 4º en que se extirpó el total de la masa esplénica.

Los pesos sumados de los 4 fragmentos fueron mayores que los de los testigos y la diferencia alcanzó significado estadístico.

El aumento de peso no debe, sin embargo, atribuirse necesariamente a hiperplasia, pudiendo deberse a simple congestión de los senos.

De cualquier manera es un hecho que luego de ninguna de las extirpaciones parciales el fragmento restante alcanzó a hipertrofiarse hasta compensar, en peso, la parte eliminada, por lo que no puede hablarse de hipertrofia compensadora en sentido anatómico.

TABLA II

Cantidades de tejido esplénico cuya extirpación permitió el desarrollo del síndrome hemolítico

Nº	Peso	Peso del bazo (absoluto)	Peso del bazo %
E 851	270	269	100
E 823	246	156	64
E 849	206	121	59
E 829	245	144	59
E 633	245	200	82
E 711	289	156	54
E 635	250	333	134

Además, la esplenectomía en 4 tiempos permitió comprobar que los animales controlaban la infección con cantidades de tejido esplénico a veces muy por debajo del 25 % del peso inicial, hasta 10 %, y que extirpada esa masa de bazo la infección se desarrollaba de la misma manera que en las esplenectomizadas en 1 tiempo, lo que permite descartar la hipertrofia funcional del resto del S.R.E., y muestra la marcada especificidad de la protección esplénica frente a la bartonella.

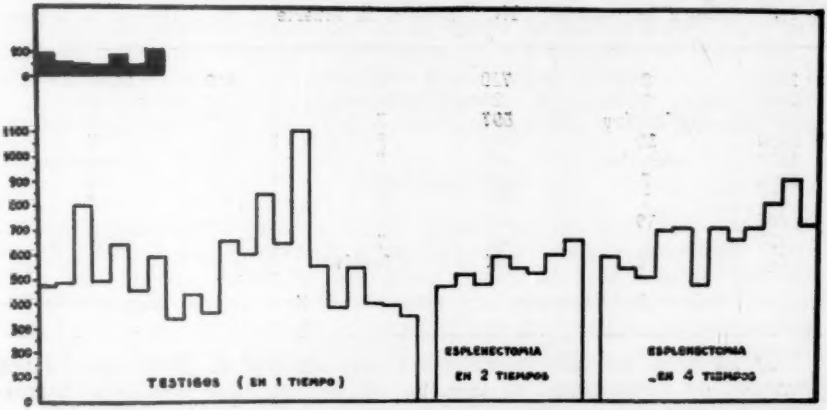

FIG. 2. — Columnas blancas: *Pesos de los fragmentos sumados en esplenectomías en 1, 2 y 4 tiempos (miligramos por cien gramos de peso).*
Columnas negras: *Pesos mínimos de bazo capaces de impedir el desarrollo del síndrome hemolítico en 7 animales (la misma escala).*

III) *Mecanismo de la defensa*

a) Los experimentos anteriores conducen a la necesidad de la permanencia en el organismo de una cantidad mínima de tejido esplénico bien irrigado. Es posible provocar el síndrome hemolítico sin esplenectomía, simplemente ligando el pedículo esplénico u ocasionando una trombosis de sus vasos, de tal modo que la necrosis del órgano exceda a esa cantidad mínima.

La protección esplénica podría atribuirse a un factor humoral producido en el bazo y presente en el suero sanguíneo; cabía por lo tanto suponer que la inyección de suero de rata normal fuera capaz de suplir la ausencia del órgano. Diez ratas esplenectomizadas recibieron por vía intraperitoneal 10 a 12 cc diarios de suero fresco de rata con bazo, y otras 10 fueron inyectadas con 10 a 12 cc del mismo suero por vía endovenosa.

El porcentaje de supervivencia, según puede verse en la Tabla III, es el 20 %, exactamente como en los animales no tratados.

El mismo resultado negativo se obtuvo inyectando por vía intraperitoneal un macerado de bazo filtrado y suspendido en solución fisiológica, en dosis equivalentes a 1 bazo diario. De 10 animales inyectados, 7 murieron entre 5 y 24 días después de la operación, y los que sobrevivieron mostraron signos clínicos y hematológicos de la infección.

TABLA III

Supervivencia de ratas esplenectomizadas inyectadas con suero de rata normal y con macerado de bazo

Suero intraperitoneal		Suero endovenoso		Macerado de bazo	
Nº	Días a la muerte	Nº	Días a la muerte	Nº	Días a la muerte
1310	8	713	5	1500	sobrevive
1240	6	84	sobrevive	1356	10
1380	sobrevive	597	5	1478	24
1488	10	879	5	1448	5
1480	sobrevive	951	7	1430	sobrevive
1392	5	1021	9	1292	9
891	8	777	7	1516	5
865	10	485	6	1266	9
491	8	998	5	1468	sobrevive
485	10	1024	sobrevive	1332	6
	Sobrevida 20 %		Sobrevida 20 %		Sobrevida 30 %

b) El bazo ha sido mencionado con insistencia como un órgano formador de anticuerpos, si bien no existen pruebas absolutas al respecto. Su acción de control de la infección bartonelósica podía obedecer a un mecanismo antigénico que al faltar el bazo quedaba súbitamente interrumpido, permitiendo el desarrollo de la infección latente.

Para comprobar el grado en que la carencia de bazo influye en la formación de anticuerpos se inyectó a esplenoprivos recientes y de muchos días de esplenectomía (esterilizados con Às) glóbulos rojos de cobayo como antígeno, y luego de 7 a 10 días se midió el título aglutinante del suero.

Título de aglutininas en el suero de esplenoprivos y normales

Esplenoprivos: 2 8 2 4 0 4 8 8 8 16 16 4 2 2 0 0 0 $x = 4.94$

Testigos: 4 8 4 4 4 4 8 8 16 16 8 8 2 0 4 4 4 4 $x = 6$

$t = 0.83$ (no significativa).

Los títulos séricos de aglutininas anti glóbulos rojos de cobayo en los animales esplenoprivos fueron iguales o muy poco menores que los testigos y la diferencia no tiene significado.

IV) *Infección de ratas con bazo*

El desarrollo espontáneo de la infección en normales con bazo es un hecho posible pero de excepción, y se observa sólo raramente en animales debilitados por otras causas. Con el fin de comprobar el grado de defensa de los normales con bazo frente a la invasión de grandes cantidades de bartonellas se inyectó a 14 ratas jóvenes glóbulos infectados, o sea provenientes de animales con síndrome hemolítico, en cantidades variables entre 1 y 3 cc de glóbulos puros, separados por centrifugación. Diez de los 14 contrajeron la enfermedad que mostró los mismos síntomas clínicos y signos hematológicos que los normales es-

plenectomizados con la particularidad de que el período de latencia habitualmente de 3 a 4 días se acortó a 1 ó 2 días.

Los que no mostraron signos de infección habían recibido cantidades de sangre iguales que los otros, por lo que la diferencia parece deberse más bien que a la cantidad a la calidad infectante de la misma, que depende del número de gérmenes circulantes en el momento de la toma de la sangre.

La autopsia mostró esencialmente las mismas lesiones que en los animales esplenectomizados, con el agregado de que la pulpa esplénica, llena de restos de hematíes fagocitados, se vió invadida, al igual que el hígado, por acúmulos de neutrófilos de distintos tamaños.

Por otra parte, las lesiones hepáticas de sobrecarga grasa e infiltración leucocitaria fueron mucho más intensas que en los esplenoprivos.

Discusión

La observación durante largo tiempo de los animales enteros y esplenoprivos que sobreviven a la infección permite reconocer en el ciclo vital de la bartonella dos fases distintas: una latente, invisible al microscopio, en la que permanece sin provocar síntomas durante toda la vida de los animales, y otra coloreable y visible al microscopio, que dura unos pocos días en cada empuje infeccioso y en la que parasita los glóbulos rojos destruyéndolos.

La presencia del bazo en los animales enteros no impide que la fase latente se prolongue durante toda la vida una vez infectado el animal, vale decir no esteriliza, como lo prueba la morbilidad del 100 % de los adultos esplenectomizados a cualquier edad.

Pero, por otra parte, la presencia del bazo tampoco impide la multiplicación de los gérmenes una vez alcanzada la fase visible, ni logra controlar su acción hemolítica, que frecuentemente conduce a la muerte del portador, hecho observado en los animales enteros inyectados con sagre de enfermos en el momento de la diseminación bacteriana.

La función protectora del bazo se ejercería entonces impidiendo la transformación de la fase latente en la hemolítica, y esta función sería específica del tejido esplénico por cuanto mientras cantidades mínimas del mismo son capaces de realizarla, la esplenectomía total va fatalmente seguida por una bacteriemia, expresión de la fase hemolítica del germen.

Parece probable entonces que para evitar el desarrollo de la infección es necesario el pasaje periódico de toda la sangre a través de los senos esplénicos, a cuyo nivel tendrían lugar los fenómenos químicos o enzimáticos que impedirían a la bartonella pasar de la fase latente a la hemolítica, haciéndose al mismo tiempo visible al microscopio.

Ahora bien: la protección esplénica no parece deberse a un mecanismo inmunitario, por cuanto es un hecho común de observación que la producción de anticuerpos no depende exclusivamente del bazo, y por otra parte nosotros hemos podido medir títulos iguales de aglutininas anti glóbulos de cobayo en esplenoprivos y normales.

Tampoco parece deberse a la producción de sustancias de tipo hormonal circulantes en el suero, en favor de lo cual está la incapacidad

del suero normal para impedir la infección. Apoya esta idea además, la observación de hembras esplenoprivas gestantes, que en los últimos días de la preñez sufren recaídas con muerte en la mayoría de los casos, sin que los bazos de sus numerosos fetos hayan podido controlar la infección materna.

Por otra parte ha sido posible observar que el bazo carece de la capacidad de hipertrofia compensadora común a otros parénquimas. Analizando los experimentos de Palmer y colaboradores ([7]) sobre la esplenectomía total y parcial en la cifra de leucocitos, es posible comprobar la ausencia de hipertrofia compensadora esplénica, pero esos experimentos se realizaron en cepas no portadoras de bartonellas, y en consecuencia en sus animales no existía la necesidad vital de la barrera esplénica al desarrollo de la infección. Los nuestros, portadores, estuvieron en el 100 % de los casos sometidos al estímulo que significa la presencia del germen con su potencial capacidad hemolítica, y no obstante ello, las esplenectomías en dos y cuatro tiempos permitieron comprobar que una vez extraída una parte importante del bazo, el resto del tejido esplénico no se hipertrofia como podría esperarse, considerando lo necesario de su presencia y la especificidad de su acción protectora.

Las lesiones histológicas observadas son inespecíficas y ninguna parece revestir una gravedad que justifique el elevado porcentaje de muertes. La alteración más importante es la intensa anemia, que podría ser responsable de la elevada mortalidad. No obstante, la observación de que animales anemizados por sangría pueden soportar cifras aún más bajas sin mayores daños arroja dudas sobre ese mecanismo de la muerte y permite establecer la hipótesis de que la misma se deba a causas ajenas a la anemia y más bien vinculadas con la hemólisis o la intoxicación bacteriana.

Es interesante hacer notar que en ningún caso los animales aún con las anemias más intensas mostraron anuria, hecho que concuerda con los cuadros histológicos renales en que no se observan las típicas lesiones de la nefrosis del nefrón inferior.

La relativa indemnidad del riñón pese a la enorme cantidad de desechos globulares ofrecidos para su eliminación, encuentra su correspondencia en la falta de lesiones renales graves en las anemias hemolíticas agudas del hombre, favismo por ejemplo, en que a pesar de la enorme destrucción sanguínea no se observan signos serios de taponamiento renal, y pone de relieve la marcada especificidad de las nefrosis del nefrón distal por incompatibilidad en los accidentes transfusionales.

Resumen

Ratas blancas infectadas con "Bartonella muris" llevan el germen en forma latente durante toda la vida sin presentar síntomas si no se les extirpa el bazo, pero luego de la esplenectomía desarrollan un síndrome hemolítico agudo con intensa leucocitosis neutrófila y muerte en el 80 % de los casos.

Es posible producir el síndrome hemolítico sin esplenectomía inyectando a animales enteros sangre infectada, es decir, proveniente de un animal enfermo en la fase aguda del proceso.

El mecanismo de la protección esplénica frente a la infección es desconocido, pero parece no tratarse de un fenómeno inmunitario, ni de la producción de sustancias de tipo hormonal.

No ha sido posible comprobar hipertrofia compensadora del bazo.

Se discuten los posibles mecanismos de la protección esplénica.

SUMMARY

Albino rats carriers of "bartonella" infection live without symptoms as long as the spleen is not removed. After splenectomy, however, they develop an acute hemolytic syndrome, accompanied by intense neutrophilia and followed by death in 80 % of the cases.

The hemolytic syndrome is also elicited in intact rats by the injection of infected blood i. e. blood taken from an animal in the acute phase of the illness.

The mechanism of splenic protection is not known, but there is evidence suggesting that it is not produced through immunological processes or hormonal substances.

There is no compensatory hypertrophy of the spleen.

Possible mechanisms of splenic protection are discussed.

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INFLUENCE OF GLUCOSE, FRUCTOSE AND HEXOSEPHOSPHATES ON GLYCOGEN BREAKDOWN AND OXYGEN UPTAKE BY RAT LIVER SLICES

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RAT LIVER slices in a glucose containing medium do not show an increased oxygen consumption, as compared to that in unfortified media (Warburg, 1928, page 137; and many others). However, an increased uptake of oxygen is brought about by addition of pyruvate (Kisch, 1934; and many others). Furthermore, liver slices evolve much less $C^{14}O_2$ when allowed to act on C^{14} labeled glucose than do other tissues (Olson, 1951). Besides, liver slices incorporate glucose of the medium into glycogen, as has been definitely demonstrated with labeled glucose (Hastings et al., 1952).

The main purpose of the present study was to determine which is the limiting stage in glucose catabolism. As the measurement of oxygen uptake showed that there was a blockade in the conversión of glucose to glucose-6-phosphate, it seemed convenient to compare the influence of glucose and hexosephosphates on glycogen formation. In addition, some experiments with fructose were performed.

EXPERIMENTAL

Male rats weighing from 200 to 250 g were used. The animals were sacrificed by a sharp blow on the head and exsanguinated thereafter. The livers were rapidly excised and placed in cold saline. Slices were prepared free-hand, with a razor blade and kept in oxygenated cold buffer solution. The slices were gently blotted on filter paper, weighed on a torsion balance and transfered to conventional Warburg vessels. Experiments were carried out in duplicate, at 37.7° C, using 2 ml of medium and about 100 mg of tissue. Krebs-phosphate solution (Krebs, 1933) modified to contain less calcium, or Hastings bicarbonate buffer

* Some of the experiments presented in this paper were performed by one of the authors (H.N.) in Dr. A. B. Hastings' Laboratory, Department of Biochemistry, Harvard Medical School, during the tenure of a Fellowship from the Guggenheim Foundation.

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(Hastings et al., 1952) were used, equilibrating with oxygen or with a 95 per cent O_2-5 per cent CO_2 mixture, respectively. The following substrates were assayed: glucose; fructose from S.A.F. Hoffmann La Roche & Cie; glucose-1-phosphate *. glucose-6-phosphate, fructose-6-phosphate and fructose-1,6- diphosphate, all from Schwarz Laboratories, Inc. G-1-P was the potassium salt; the barium salts of the other hexosephosphates were converted to sodium or to potassium salt, respectively, when Krebs or Hastings solution were used. The substrates were added to the saline, replacing appropiate amounts of NaCl or KCl by equiosmolar solutions of the substances, to obtain a 30 mM/L final concentration.

After ten minutes of thermal equilibrium one vessel from the triplicate of each experimental condition was removed and tissue glycogen was determined. This is referred as "initial glycogen". After 60 minutes of incubation the tissue's "final glycogen" was measured in the two other vessels. Glycogen was determined by the procedure of Walaas and Walaas (1950) with Somogyi (1945) and Nelson (1944) reagents. Oxygen uptake was measured in the usual way and expressed as microliters taken up per 100 mg wet weight of tissue per hour.

RESULTS

Influence of different substrates on oxygen consumption. — Table I shows that fructose and hexosephosphates increase oxygen consumption by rat liver slices, with variable intensity, according to the substrate used, while glucose does not.

TABLE I

Effect of different substrates on oxygen uptake by rat liver slices *

Substrates	N° of Experiments	0_2 uptake **
Glucose	40	+ 1.2 ± 0.7
Fructose	20	+ 18.9 ± 2.1
Glucose-1-phosphate	18	+ 9.6 ± 1.4
Glucose-6-phosphate	18	+ 13.1 ± 1.1
Fructose-6-phosphate	14	+ 18.3 ± 2.5
Fructose-1, 6-di-phosphate	15	+ 23.3 ± 2.0

* Some of the experiments included in this table have been briefly reported (Niemeyer, 1951; Niemeyer et al., 1953).

** Arithmetic mean and standard error of the per cent change of oxygen uptake determined by the addition of substrates at 30 mM/L.

Effect of different substrates on liver glycogen. — The results concerned with the effect of glucose, fructose and their phosphoric esters on liver glycogen were rather surprising, as it is shown on Ta-

* G-1-P will be used to designate glucose-1-phosphate; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-di-P, fructose-1, 6-diphosphate.

TABLE II

Comparison of the effect of different substrates on glycogen content of rat liver slices *

Substrate 1	Substrate 2	Nº of Experiments	Difference **	P ***
		Krebs' Medium		
Fructose	Glucose	13	+ 31.8 ± 4.3	< 0.001
Glucose	G-6-P	14	+ 16.4 ± 3.2	< 0.001
G-6-P	None	14	+ 9.4 ± 2.9	< 0.01
F-6-P	None	11	+ 1.6 ± 2.2	< 0.4
F-di-P	None	9	− 6.3 ± 8.1	< 0.4
		Hastings' Medium		
Glucose	G-6-P	8	+ 45.0 ± 16.0	< 0.03
Glucose	G-1-P	11	+ 62.6 ± 12.6	< 0.001
Glucose	None	14	+ 68.6 ± 14.3	< 0.001
G-1-P	None	8	+ 6.4 ± 8.8	< 0.4

* Some of the experiments included in this table have been briefly reported (Niemeyer et al., 1953; Cruz Coke et al., 1954).

Final glycogen (G_f) without added substrate was 24.8 ± 2.2 per cent of initial glycogen (G_i) when Krebs' solution was used as the incubation medium. The respective value for Hastings' solution was 75.9 ± 5.0 %.

** To compare the effect of two substrates (1 and 2), slices from the same liver were used and G_i and G_f in both conditions were determined. Difference, used as a criterion of comparison, was calculated according to the following formula:

Difference $= 100\ (G_{f1} - G_{f2})/G_i$. The figures of the table correspond to the arithmetic mean and its standard error.

*** P values were deduced from t = mean/S.E.

ble II. Glucose inhibited glycogen breakdown of slices incubated in Krebs-phosphate solution significantly more than G-6-P. In the same medium, the effect of fructose was higher than that of glucose, while F-6-P and F-di-P did not interfere with glycogen breakdown. When Hastings' solution was used as the incubation medium, glucose usually determined a net synthesis of glycogen. Although G-6-P in some cases produced a net synthesis its effect was significantly less than that of glucose. G-1-P did not inhibit glycogen breakdown at all.

Effect of inorganic phosphate concentration in the incubation medium. — Previous experiments had shown that the increase of phosphate in the medium up to 32 mM/L did not modify the oxygen uptake by rat liver slices incubated with or without glucose (Niemeyer et al., 1953). Table III shows that increasing phosphate concentration up to

TABLE III

Influence of inorganic phosphate of the medium on glycogen evolution in rat liver slices incubated with glucose or without substrate

Exp.	Initial Glycogen	Final Glycogen (%)*					
		With Substrate			With Glucose (30 mM/L)		
Nº	%	8	16	24	8	16	24**
1	0.42	—	0.07	0.07	—	0.08	0.08
2	—	0.75	—	0.75	1.25	1.20	1.45
3	—	0.81	0.96	0.86	1.76	1.46	1.56
4	3.10	—	1.24	1.19	—	1.69	1.59
5	4.49	—	1.31	1.22	—	1.84	1.87
6	7.50	—	3.54	3.62	—	5.19	5.15

* Glycogen content after one hour of incubation.

** These figures indicate the concentration of inorganic phosphate in the medium (mM/L).

24 mM/L neither changes glycogen breakdown rate in the absence of substrates, nor modifies the effect of glucose on this process.

Discussion

The results presented above indicate that fructose and the hexosephosphates increase oxygen uptake, while glucose does not. These results lead to the conclusion that the conversion of glucose to G-6-P is a limiting step in the catabolism of glucose. This conclusion is supported by the finding that the aerobic extraproduction of lactic acid by rat liver slices is higher with G-6-P than with glucose as a substrate: 1.08 ± 0.16 versus 0.62 ± 0.06 μM per 100 mg wet tissue per hour (Niemeyer, González & Figueroa, 1956).

However, we found, in opposition to what we expected, that glucose forms glycogen at a higher rate than G-6-P and G-1-P in liver slices incubated in two different media. Under the same conditions G-6-P is a better precursor than G-1-P in glycogen formation. These results are not easily explained on the basis of present knowledge on glycogen synthesis. They cannot be the consequence of the excess of phosphate released to the medium by phosphatase action upon hexosephosphates (Niemeyer et al., 1954). In fact, the increase of inorganic phosphate up to 24 mM/L, a figure which far surpasses the concentration reached in the experiments performed with hexosephosphates, did not interfere in glycogen breakdown.

It seems useful to discuss briefly the mechanisms by which glucose would contribute to glycogen synthesis in liver. The present schemes concerned with carbohydrate metabolism contend that glucose is first converted to G-6-P through glucokinase reaction. This would bring

about an increasing amount of G-6-P, which would then be converted to glycogen, through G-1-P as intermediate. Glucose may also interfere in glucose-6-phosphatase reaction by mass action, restricting G-6-P dephosphorylation. This possibility is supported by the studies of Meyerhof and Green (1949), who proved the reversibility of phosphatase action. We were able to test this assumption in our experimental conditions. In fact, we found that the presence of glucose (30 mM/L) inhibited the release of inorganic phosphate from G-6-P (30 mM/L) by rat liver slices to the extent of 17 ± 3.7 %. Both of these mechanisms, which ultimately lead to an increase in the intracellular level of G-6-P, do not explain the difference between the effects of glucose and G-6-P on liver glycogen. Indeed, we must accept that a higher intracellular level of the hexosephosphate is obtained with G-6-P than with glucose as a substrate. Our experimental data, which show a higher oxygen consumption and a higher lactic acid production in the presence of G-6-P, lend support to this contention.

As it has been shown that glucose inhibits phosphorylase, it has been assumed that glucose might block glycogen breakdown through this pharmacological action, which is not shared by fructose or hexosephosphates (Lehmann, 1938; Gill & Lehmann, 1939; Cori et al., 1939; Cori & Cori, 1940; Cori et al., 1943). This action may well be invoked to explain why the final glycogen content of slices incubated in Krebs' solution —an experimental condition in which reactions proceed predominantly in the direction of breakdown— remains at a higher level with glucose than with G-6-P as a substrate. The mere fact that a net synthesis of glycogen occurs in slices incubated in Hastings' solution with glucose, makes it difficult to accept a role of the above mentioned pharmacological action, if glycogen synthesis is dependent on the action of phosphorylase. Besides, 2.4-dinitrophenol, that prevents the oxidative generation of high energy phosphate bonds, inhibits glycogen synthesis from glucose almost completely under certain circumstances, an effect which is not explained assuming that free glucose acts as a pharmaco (Niemeyer, Figueroa & González, 1956).

The analysis of the possible mechanisms through which glucose contributes to the synthesis of glycogen indicates that presente knowledge does not offer an explanation for our experimental findings. This has induced us to consider the possibility that glycogen might be formed from glucose through another pathway, that excludes G-6-P and G-1-P as intermediates. The role of these esters would be confined chiefly to glycogen degradation. Thus, the small effect of G-6-P on glycogen breakdown would be the consequence of its conversion to free glucose by glucose-6-phosphatase. The higher glycogenetic effect of G-6-P as compared with G-1-P may be explained by the fact that the former compound liberates inorganic phosphate and glucose at a higher rate (Niemeyer et al., 1954).

The hypothesis of a new pathway for glycogen synthesis from glucose accounts for the following observations:

1. Hexokinase activity is considerably lower in liver than in any other rat tissue (Long, 1951) and, on the other hand, glucose-6-phosphatase activity is very high (Hers & Duve, 1950; Niemeyer et al., 1954).

2. The presence of L-sorbose-1-phosphate and DL-gliceraldehyde, which inhibit hexokinase (Lardy et al., 1950) does not affect glycogen formation from glucose in rat liver slices (Enrique Figueroa et al., 1956). 3. Floridzin, which inhibits phosphorylase (Cori et al, 1943), does not prevent synthesis of glycogen from glucose in liver slices suspended in Hastings' solution (Cruz Coke et al., 1954). 4. Epinephrine and hyperglicemic-glycogenolytic factor, which promote the syntesis of active phosphorylase, stimulate the breakdown of glycogen, but not its synthesis, both in liver slices and in the whole animal (Sutherland, 1951).

The idea that glucose monophosphates are intermediates chiefly in glycogen breakdown, is quite understandable if one considers the thermodinamical conditions of the reactions: glycogen $\rightleftarrows$ G-1-P $\rightleftarrows$ G-6-P $\rightleftarrows$ glucose.

Firstly, the concentration of inorganic phosphate in the liver of fed rats and fasted rats is 17.8 ± 0.6 and 29.3 ± 0.6 mg per 100 grams respectively (Rapoport et al., 1943). It seems most probable that these concentrations, which correspond approximately to 7.2 and 12.0 mM per liter of liver water, favour the conversion of glycogen to G-1-P, in view of the data obtained in experiments performed in isolated enzyme systems (Sutherland et al., 1941). In second place, the equilibrium of the reaction catalized by the phosphoglucomutase favours the formation of G-6-P from G-1-P (Sutherland et al., 1941). Finally, these reactions must be shifted to the right because, as it has already been pointed out, in the liver the activity of glucose-6-phosphatase is very high, whilst that of glucokinase is extremely low.

The results concerned with the effect of fructose on liver glycogen also suggest that the hexosephosphates assayed are not intermediates in the pathway from fructose to glycogen.

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SUMMARY

The influence of glucose, fructose and several phosphorylated hexoses on oxygen uptake and glycogen content of rat liver slices was studied. Glucose does not modify the basal oxygen uptake, whilst fructose and phosphorylated hexoses increase it from ten to twenty per cent.

On the other hand, glucose inhibits glycogen breakdown or promotes glycogen synthesis much more than glucosephosphates.

These results, alongside those collected from the literature, are discussed and the suggestion is made that the formation of glycogen from glucose, in the liver, proceeds by a pathway that excludes glucophosphates as intermediates.

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ACTION OF CORTISONE ON THE RIBONUCLEIC ACID AND PROTEIN NITROGEN CONTENT OF THE ADRENAL GLAND

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IT HAS BEEN shown that cortisone (17-hydroxi, 11-dehydro-corticosterone) is an inhibitor of the compensatory hypertrophy of the rat adrenal gland when the animal is unilaterally adrenalectomized ([5]) and that cortisone is related to protein catabolism ([4]), although it stimulates the protein synthesis in the liver ([8]).

In previous reports from this laboratory, Villela and Povoa ([9], [10]) have established the values for the ribonucleic acid (RNA) content for the normal adrenal glands and for those with compensatory hypertrophy. In this last condition, no increase for the RNA content of the hypertrophied gland was found. The only work about RNA in adrenal gland we have found in the litterature was that of Goldshtein et al. who reported an increase of the RNA content of guinea pig adrenal glands in vitamin C deficiency ([2]).

In the present work, the effect of cortisone on the RNA and protein nitrogen content of the adrenal glands was studied.

MATERIAL AND METHODS

Wistar white rats were used throughout this study. After a slight ether anesthesia, the animals were operated and the left adrenal gland rapidly excised, blotted and grinded with celite in a centrifuge tube. The right gland was removed at intervals varying from one to ten days after the operation.

The adrenal glands from 2 to 3 rats were pooled and the RNA extracted according to the technique of Schmidt and Thanhauser ([6]). After homogenization with cold 10 % trichloracetic acid, they were successively extracted with cold 1 % trichloracetic acid, water, ethanol, ethanol-ether and the residue finally dried with ether and hydrolized with 2.5 ml N KOH for 17 hours. The desoxiribonucleotides were precipitated with an acid mixture of 6 N HCl and 10 % trichloracetic acid (1:3). The ribonucleotides were twice extracted with this acid

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mixture and the final volume was completed to 7 ml. An aliquot of 1 ml was taken and heated with equal volume of orcinol-hydrochloric acid-ferric chloride reagent, in a water bath, at 100° C, for 45 minutes, according to the technique of Mejbaum ([3]). The color was read at 650 millimicra in a photocolorimeter and the results expressed as micrograms of ribose or RNA.

TABLE I

RNA variation in adrenalectomized rats

(The first rows represent the data obtained from rats before the operation and the second ones from those after unilateral adrenalectomy)

Day interval between the two operations	Average weight of the rats (g)	Average weight of the adrenals (mg)	Ratio: Adrenal weight/ rat weight (µg/g)	RNA (mg/100 g of tissue)	RNA (µg/ total gland)	Per cent variation µg/total gland)
1	99	9.6	96	894	85.8	— 43.1
	99	10.8	109	452	48.8	
2	130	14.4	110	1552	223.0	— 43.0
	134	16.2	120	783	127.0	
2	140	14.3	102	569	81.5	+ 72.5
	129	17.8	137	790	140.6	
3	278	20.4	70	930	189.0	— 6.4
	281	23.4	80	725	177.0	
7	119	15.3	128	967	148.0	— 52.0
	127	16.6	130	427	71.0	
9	87	12.6	144	2156	273.4	— 48.6
	102	13.3	130	1053	140.6	
10	153	13.4	87	665	88.8	+ 58.3
	157	17.0	108	829	140.6	

Protein nitrogen content was determined in the residue by the micro-kjeldahl technique previously described ([1]), after a duplicate extraction of the desoxiribonucleotides with 5 % trichloracetic acid, during 15 minutes, at 90-95° C, according to the method of Schneider ([7]).

Results and discussion

Our results were expressed in Tables I, II, III and IV and Fig. 1. In Tables I and II, the first rows represent data from rats before the operation and the second one from those after unilateral adrenalectomy. Table III shows the variation of protein nitrogen content of the whole

TABLE II

RNA variation in adrenalectomized rats treated with cortisone

(The first rows represent the data obtained from rats before the operation and the second ones from those after unilateral adrenalectomy)

Day interval between the two operations	Average weight of the rats (g)	Average weight of the adrenals (mg)	Ratio: Adrenal weight rat weight (μg/g)	RNA (mg/100 g of tissue)	RNA (μg/ total gland)	Per cent variation μg/total gland)	Cortisone daily dosis (mg)	Cortisone total dosis (mg)
1	89.5	11.2	125	1392	156.0	+ 22.1	7.5	7.5
	87.5	12.6	144	1549	190.5			
1	144.5	11.0	76	781	85.6	+ 7.9	7.5	7.5
	141.0	12.7	90	724	92.4			
2	98.0	10.7	109	863	92.4	+ 8.2	2.5	5.0
	95.0	13.4	141	772	104.0			
2	136.5	12.1	88	1054	127.0	+ 63.7	2.5	5.0
	133.0	14.7	110	1409	208.0			
3	109.5	10.6	96	813	85.6	+ 61.9	3.3	10.0
	101.5	9.8	95	1414	138.6			
7	124.0	12.3	99	1445	179.0	0	1.4	10.0
	109.0	12.3	112	1414	179.0			
9	78.5	11.6	147	1586	184.8	+ 3.1	2.2	20.0
	73.0	8.6	117	2202	190.5			
10								
	103.0	10.3	100	841	86.6	+ 66.5	1.7	17.5
	103.5	8.5	82	1688	144.3			

gland due to the compensatory hypertrophy and of the cortisone-treated rats after unilateral adrenalectomy. Table IV shows the RNA content of the left and right adrenal glands in the same animals and Fig. 1 the average RNA and protein nitrogen content (micrograms/whole gland) in rats treated and not treated with cortisone after unilateral

TABLE III

Protein nitrogen variation in adrenalectomized rats not treated and treated with cortisone

Day interval between the two operations	Protein nitrogen variation (µg/whole gland)				Cortisone daily dosis (mg)	Cortisone total dosis (mg)
	Cortisone-untreated	Per cent variation	Cortisone-treated	Per cent variation		
1	47.1-51.8	+ 10.0	65.0-68.9	+ 6.0	7.5	7.5
2	79.9-83.1	+ 4.0	65.2-72.1	+ 10.6	2.5	5.0
3	53.9-54.1	+ 0.4	63.5-70.3	+ 10.7	3.3	10.0
3	51.8-55.3	+ 6.7	55.0-60.5	+ 10.0	3.3	10.0
7	77.7-80.0	+ 0.4	55.0-68.5	+ 24.5	1.4	10.0
9	48.8-51.8	+ 6.2	55.0-60.5	+ 10.0	2.2	20.0
Average mean of the increase	3.8	5.2	7.0	11.9		

adrenalectomy. From the data tabulated (Table I), it is clearly shown that the RNA content of the hypertrophied glands was unchanged. An average diminution of 34.8 micrograms (Fig. 1) was observed which is not statistically significant ($0.4 < p < 0.3$). The RNA content referred for the whole gland was increased in cortisone-treated rats (Table II). This increase was of statistical significance ($p < 0.02$). Protein

TABLE IV

RNA content of left and right adrenal glands
(Same animals)

	Number of glands	Average weight of the glands (mg)	RNA/100 g (mg)	RNA/total gland (µg)
Right	2	19.0	563.0	214.0
Left	2	16.2	643.0	208.0

nitrogen content of adrenal glands in cortisone-treated as compared with cortisone-untreated rats, after unilateral adrenalectomy (Table III), showed also an increase statistically significant ($p < 0.05$). RNA content calculated for the total gland (left and right) presented similar values although these values were different when calculated per 100 g of tissue (Table IV).

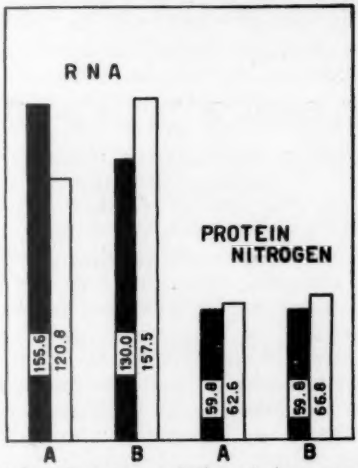

FIG. 1. — *RNA and protein nitrogen variation (micrograms/whole gland). The first colums (black) represent the average data before the adrenalectomy and the second ones (white) those after this operation.*
A — *Cortisone-not treated animals.*
B — *Cortisone-treated animal.*

SUMMARY

The RNA and protein nitrogen content of rat adrenal glands were determined before and after compensatory hyperthophy (by unilateral adrenalectomy) and in cortisone-treated animals in the same conditions. The results showed an increase of RNA and protein nitrogen which is statistically significant.

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STIMULATING EFFECT OF ACETYLCHOLINE ON THE PAPILLARY MYOCARDIUM *

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ON THE BASIS of previous work carried out in this laboratory, a hypothesis was advanced that either in the heart itself or in its proximity there are ganglionic cells which, under the action of vagal preganglionic impulses ([10]) or of injected acetylcholine ([7]), release an epinephrine-like substance which stimulates cardiac activity.

However, the interpretation that the cardiostimulating action of acetylcholine requires the presence of ganglionic cells is open to doubt since it is possible that this transmitter may act exclusively (or as well) by mechanisms which do not involve the activation of these elements.

An analysis of the action of acetylcholine on myocardium deprived of ganglionic structures could clarify this problem. Current morphological evidence indicates that ganglionic cell occur only rarely in the ventricles ([12]). Since there they are predominantly located immediately under the epicardium ([8]), it appeared likely that in the ventricular papillary muscles there are no ganglionic cells at all.

In the present work we have made a study of the microscopical structure of the papillary muscles of the cat's heart. We failed to find in them any ganglionic cells or chromaffine tissue. It seems, therefore, that the papillary muscle provides, in fact, a preparation without ganglionic cells and, consequently, an analysis of the action of acetylcholine on this tissue appeared to be of special interest.

METHODS

The staining techniques used in the microscopical study of the papillary muscles, will be referred to when the results are described. For the analysis of myocardial reactions, papillary muscles from the cat's right ventricle were used. The muscles were removed from the animal

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* A preliminary report on this work was communicated at the XIX International Congress of Physiology (1953, Montreal, Canadá).

Received for publication, December 27th, 1955.

under Dial anesthesia (0.08 g/Kg, intraperitoneally), placed in a moist chamber and irrigated drop by drop with Tyrode solution warmed to 37° C and saturated with 95 % O_2 and 5 % CO_2. This technique is similar to that described by Gaddum et al. ([6]) for the surviving rectal cecum of the fowl. By means of a special devise, single doses of acetylcholine and other agents were added to the irrigating fluid in the immediate proximity of the muscle, without altering the frequency of the drops or the temperature of the fluid. Several experiments were conducted on muscles immersed in Tyrode solution, according to the procedure described by Cattell et al. ([3]); no qualitative differences were observed in the reactions of these muscles as compared with those irrigated drop by drop. Myocardial contractions were recorded on a smoked drum with a light semi-isometric lever. The muscles were made to contract by supramaximal condenser discharges, at frequencies varying from 50 to 75 shocks per minute. Within this range, the varying rates of contractions were without any appreciable effect on the quality of the myocardial response.

Results

Microscopical structure of the papillary muscle

Nineteen muscles from nineteen hearts were studied. Seven muscles were fixed with Orth's fluid and stained with the usual hematoxylin-eosin technique. 900 serial sections from these muscles were examined. Neither ganglionic cells, nor chromaffine tissue were observed in any of the sections. The myocardial tissue showed the structural characteristics typical of the species. Two muscles were fixed with Bouin-Hollande; 350 serial sections were stained with Heidenhain's ferric hematoxylin. The microscopical observation failed to reveal the presence of specific myocardium (Purkinje tissue). Ten muscles were fixed with 10 % or 40 % formaldehyde and impregnated with silver according to Bielschowsky, de Castro, or Gross. In all the numerous serial sections of these muscles it was possible to demonstrate the presence of bundles of fine nerve fibers which ramified abundantly and, after a rather tortuous course ended in expanded terminals in contact with the myocardial fibers. No intracellular nerve endings were demonstrable. Nerve fibers with a beaded appearance, similar to those of the autonomic efferents, were also observed.

Action of single doses of acetylcholine

a) *Not-atropinized muscles.* — In most of the 45 papillary muscles studied, acetylcholine in doses of 0.05 - 500 µg produced a decrease of the amplitude of contractions with small doses and an increase with larger doses (Fig. 1). In several muscles, acetylcholine exerted purely stimulating effects, while in others purely depressor effects were obtained. Finally, in a few preparations, no effects of acetylcholine were observed, even though the doses applied fluctuated within a wide range. The amplitude of both the depressor and the stimulating effects of the same dose of acetylcholine differed widely for the various muscles.

However, in the same muscle the stimulating effects were usually proportional to the dose applied. The depressor responses, on the other hand, were not regularly dependent on the concentration of the drug.

b) *Atropinized muscles.* — In a series of 19 muscles, atropine sulphate (2 mg/l) was added to the irrigating fluid. In all atropinized

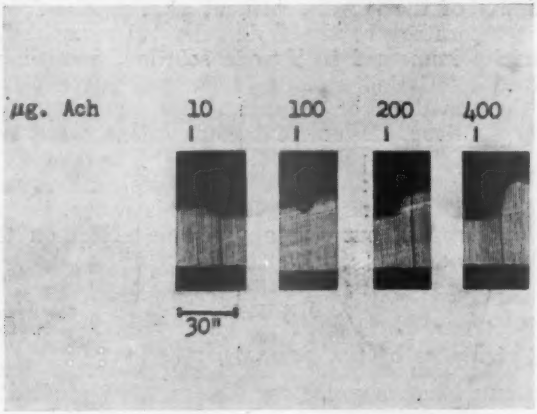

FIG. 1. — *Not-atropinized papillary muscle. Cat. Effect of single doses of acetylcholine (Ach).*

muscles acetylcholine exerted only stimulating effects regardless of the reaction induced before atropinization. Thus, if the contractions of a muscle before atropinization showed a decrease in amplitude with the application of acetylcholine, during irrigation with atropine sulphate the effect of acetylcholine was reversed. If acetylcholine had a stimulating effect on a not-atropinized muscle, this effect was always augmented by atropinization. Finally, under the effects of atropinization, stimulating effects appeared in muscles which, before atropinization, did not react to acetylcholine.

c) *Influence of ganglionic blocking agents.* — Since results of previous work ([7]) had shown that nicotine suppresses the stimulating effects of acetylcholine in the isolated heart, it seemed of interest to investigate whether this and other ganglionic blocking agents influence the effect of acetylcholine on the isolated papillary muscle.

In *not-atropinized muscles* under the action of prolonged treatment with ganglionic blocking agents, acetylcholine exerted regularly depressor effects, regardless of its previous action. Thus, nicotinization reversed the stimulating effect of acetylcholine in 15 not-atropinized papillary muscles. Fig. 2, A, shows the results of one of these experiments, in which 100 µg of acetylcholine produced a discrete increase in the amplitude of the myocardial contractions. Prolonged irrigation of the muscle with 2 mg/l of nicotine bitartrate induced initially a progressive increase of the myocardial contractions which then gradually decreased

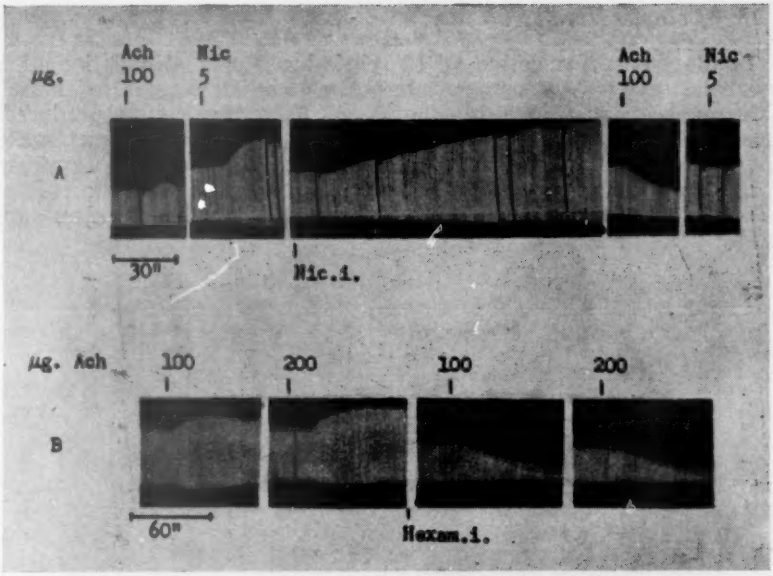

FIG. 2. — *Not-atropinized papillary muscles. Cat.*
Influence of ganglionic blocking agents on the effect of single doses of acetylcholine (Ach) and nicotine (Nic).
Nic. i., irrigation with nicotine bitartrate (2 mg/l).
Hexam. i., irrigation with hexamethonium bromide (1 mg/l).

until the muscle contracted again with a constant amplitude. In this "nicotinized" muscle, 100 µg of acetylcholine had a marked depressor effect. Similar results were obtained with other ganglionic blocking agents like tetraethylammonium (50 mg/l) and hexamethonium (0.25 - 1 mg/l); the effect of the latter is shown in Fig. 2, B.

Ganglionic blocking agents also increased already existing depressor effects of acetylcholine and made them appear when, before atropinization, this substance had not modified myocardial activity.

In seven *atropinized muscles* hexamethonium bromide, in concentrations of 0.25-1 mg/l, regularly suppressed the stimulating effects of acetylcholine. Fig. 3, shows a typical example. 100 µg of acetylcholine had a positive inotropic effect prior to the application of hexamethonium. While the preparation was being irrigated with hexamethonium, acetylcholine (100 µg) was again applied. However, no change in cardiac activity was observed, i. e., the stimulating effect of acetylcholine had been suppressed by the ganglionic blocking agent.

A similar suppressing action could be also regularly demonstrated for nicotine bitartrate (2-5 mg/l), for tetraethylammonium bromide (50 mg/l) and for acetylcholine chloride (20-75 mg/l). It is noteworthy that the blocking effect of acetylcholine was easily reversible.

Action of single doses of nicotine

Single doses of nicotine (5-10 μg) induced only stimulation of the myocardial activity, both in not-atropinized and in atropinized muscles. In the experiment shown in Fig. 2, A, 5 μg of nicotine produced a marked increase of the amplitude of the myocardial contractions. It is of interest that this stimulating effect of nicotine was suppressed by irrigation of the muscle with 2 mg/l of the same substance. It will be recalled that the stimulating action of acetylcholine is, also, supressed by nicotinization.

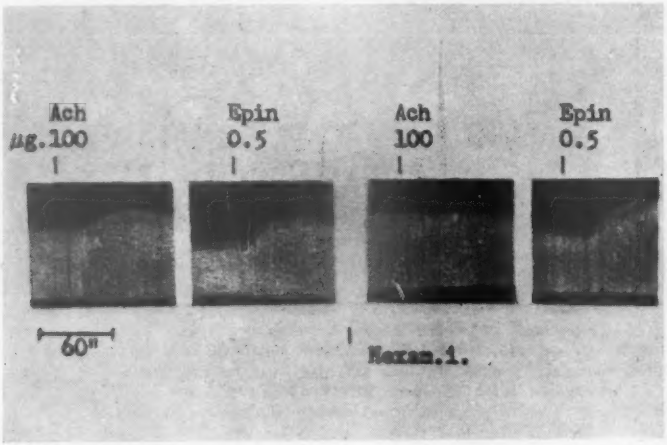

FIG. 3. — *Atropinized papillary muscle. Cat.*
Influence of ganglionic blocking agents on the effect of single doses of acetylcholine (Ach) and epinephrine (Epin).
Hexam. i., irrigation with hexamethonium bromide (1 mg/l).

Epinephrine, norepinephrine and Ca *

Single doses of epinephrine and norepinephrine (0.5-5 μg) regularly resulted in a increase of the amplitude of the myocardial contractions. The records in Fig. 4 show these effects. It may be pointed out that in this experiment norepinephrine exerted stronger stimulating effects than epinephrine. The stimulating effects induced by these substances, were not suppressed by nicotine or any other of the ganglionic blocking agents used (Fig. 3).

Calcium chloride, in doses of 100-1000 μg, had also stimulating effects, which were not modified by ganglionic blocking agents.

DISCUSSION

The results indicate that acetylcholine affects the amplitude of the contractions in a variable manner in not-atropinized myocardium. In some muscles, the amplitude of the contractions diminishes, in others

an increase is noted, while still in others no effect of acetylcholine is demonstrable. These findings contrast sharply with the effects observed in atropinized muscles. Here acetylcholine always increases the amplitude of contractions. Furthermore, in the presence of ganglionic blocking agents acetylcholine exerts only depressor effects in not atropinized preparations. These results suggest that acetylcholine exerts simultaneously both a depressor and a stimulating effect on the papillary

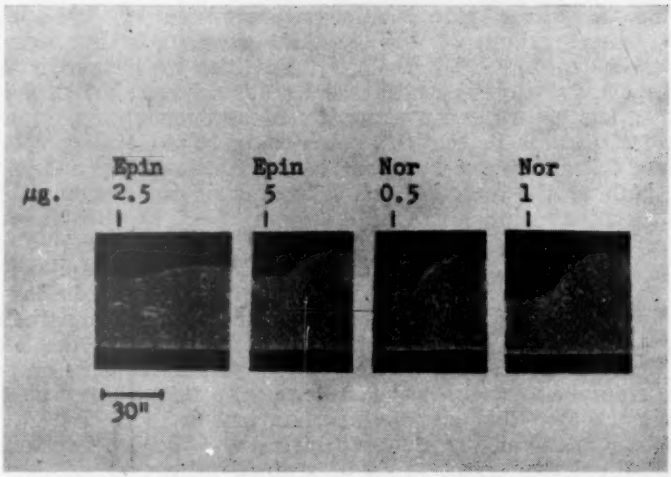

FIG. 4. — *Papillary muscle. Cat.*
Effect of single doses of epinephrine (Epin) and norepinephrine (Nor).

myocardium. Atropine, suppressing the depressor component, would unmask the stimulating action. In the presence of ganglionic blocking compounds, which inhibit the stimulating effects, acetylcholine could be expected to regularly decrease the amplitude of myocardial contractions.

In the not-atropinized isolated heart, acetylcholine exerts regularly depressor effects (7), which contrast with the irregularity of the response of the not-atropinized papillary myocardium. Further work is needed to elucidate the factors responsible for this difference. When atropine is present, however, acetylcholine acts as a stimulating agent in both the heart and papillary myocardium.

In our previous work (7) we have advanced the hypothesis that in an isolated heart the stimulating effect of acetylcholine is due to the activation of adrenergic juxta and or intracardiac ganglia. The present results, however, suggest that the papillary myocardium is devoid of ganglia and chromaffine tissue. If this be the case, the stimulating action of acetylcholine would have to be mediated through mechanisms not involving the activation of ganglionic neurons, i. e., by "non-ganglionic" mechanisms.

One of such "non-ganglionic" mechanisms could be the axon reflex. It is an intriguing phenomenon, indeed, that the "non-ganglionic" stimulating action of acetylcholine is suppressed by ganglionic blocking compounds. Coon and Rothman ([3]) have shown that nicotine and acetylcholine, injected intradermally in man and cats, induce pilomotor, sudomotor, and vasoconstrictor responses in the skin. Recently, Ambache ([1]) has obtained pilomotor reactions by intradermal injections of 3-bromophenyl-ether of choline bromide —a substance with "nicotinic" activity. These "non-ganglionic" skin reactions are abolished by local application, in high concentrations, of the same ganglionic blocking agents that in low concentrations ellicit them ([4]), and also by hexamethonium ([1]). All these effects may be considered as due to axon reflexes initiated by stimulation of branching terminals of autonomic nerve fibers that innervate structures in the skin. According to this interpretation, nicotine and other substances appear to exert stimulating and paralizing effects, both at the ganglion cell level and at the nerve endings. It follows then that suppression of a certain effect by substances of the ganglionic blocking type, does not necessarily imply that the effect is due to a stimulation of ganglionic cells. Since in the papillary muscle of the cat there are numerous nerve endings, the mediation of the stimulating effects of acetylcholine and nicotine by sympathetic axon reflexes —of a similar type as the ones responsible for the skin reactions— appears to be an attractive possibility. Experiments on papillary muscles from hearts with degenerated sympathetic innervation could determine whether this interpretation is tenable.

Another "non-ganglionic" mechanisms by which acetylcholine could exert its stimulating effect would be a direct release of epinephrine-like substances in the myocardial cells. So far, there is no clue whether acetylcholine acts on the papillary muscle by releasing epinephrine-like substances (as has been shown for the isolated heart) or not. It is important to observe that according to several authors ([2], [5], [11]), there are normally stored in the myocardium substances of the epinephrine-like type.

It should be stressed, however, that even if acetylcholine does, in fact, stimulate myocardial activity in the papillary muscle by "non-ganglionic" mechanisms, ganglionic mechanisms operating in the isolated heart would not be thereby excluded.

As is the case for the isolated heart ([7]), single doses of nicotine have a stimulating effect on the papillary myocardium. This effect resembles that of acetylcholine, since it is also suppressed by prolonged treatment with ganglionic blocking agents. As previously demonstrated ([7]), the stimulating effect of nicotine on the isolated heart is concomitant with the release of an epinephrine-like substance. However, whether an adrenergic mechanism operates in the papillary muscle, cannot be ascertained on the basis of the available evidence.

It should be noted that recently Meyling et al. ([9]), have described a system of nerve cells and fibers located peripherally to the classical post-ganglionics. This system requires special staining techniques and could easily have remained unobserved in our material. If these cells are indeed real ganglionic elements, the possibility that the stimulating

effects of acetylcholine is mediated through their activity will have to be considered.

It shoud be pointed out that in our experiments, the papillary myocardium was kept under conditions of saline and oxygen supply, which are different from those prevailing for the heart "in situ" or for one isolated and perfused with saline solution. Whether and to what extent these differences in the technique may be responsible for some of the results observed must be left an open question.

SUMMARY

In not-atropinized papillary muscles (cat), single doses of acetylcholine exert a variable effect on different preparations. Thus, the amplitude of myocardial contractions may be either decreased, increased or not changed. The effect, however, on a given muscle is constant. In most of the muscles small doses depress, while larger doses stimulate myocardial activity.

In atropinized papillary muscles, depressor effects of acetylcholine appear suppressed, and only stimulating effects are obtained. These are abolished by ganglionic blocking agents.

In muscles under the action of ganglionic blocking agents, only depressor effects of acetylcholine are observed.

These facts seem to indicate that acetylcholine produces in the papillary myocardium: a) a depressor effect which is suppressed by atropine; b) a stimulating effect which is abolished by ganglionic blocking agents.

Microcospical studies have failed to reveal the presence of ganglionic cells or chromaffine tissue in the papillary muscle. Therefore, the stimulating action of acetylcholine is interpreted as being mediated by mechanisms which do not involve activation of structures of this type. The nature of these "non-ganglionic" mechanisms is discussed in the light of results of our previous work and evidence obtained by others.

Single doses of nicotine exert exclusively stimulating effects, which are abolished by prolonged treatment with nicotine itself and other ganglionic blocking agents.

Epinephrine, norepinephrine and Ca * have stimulating effects, which are not modified by ganglionic blocking agents.

*We wish to acknowledge the collaboration of Miss Daisy Benítez and the technical assistance of Mr. Erasmo Madrid.

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Buenos Aires, April 5th, 1956

Effect of 2,4-dinitrophenol on the kidney weight and blood pressure of the rat. E. M. KRIEGER. *Institute of Experimental Physiology, Faculty of Medicine, Porto Alegre, Brazil.*

The administration of thyroid powder *per os* (30 mg/rat/day) to normal rats during 21 days causes only a slight increase in blood pressure. On the other hand, the same dose of thyroid administered to rats with reduction of the renal mass (ligature in 8 of one or both kidneys) causes a definite rise in blood pressure more accentuated the greater the dregee of renal mass reduction.

The administration of 2,4-dinitrophenol (8 mg/rat/day. intraperitoneally) has no renotrophic effect and causes no change in the blood pressure of normal rats or rats with reduction of renal mass. These findings are in favor of the hypothesis that the rise in pressure caused by the thyroid hormone is related to its renotrophic action and not to its action on oxygen consumption which is shared by 2,4-dinitrophenol.

Diabetogenic effect of Prednisone and Prednisolone in dogs and rats. J. C. PENHOS AND A. F. CARDEZA. *Institute of Experimental Biology and Medicine, Costa Rica 4185. Buenos Aires, Argentina.*

Diabetes of temporary type was induced in 50 % of the dogs with the pancreas surgically reduced to approximately 20 % of its mass, by administration of Prednisolone (*per os* or by injection) and Prednisone *(per os)*.

Hyperglycemia disappeared in 2 of these dogs in spite of continuing the treatment and, in others, when discontinuing it. Transitory diabetes in these dogs was not parallel to any toxic phenomena.

A moderate hyperplasia of islets and β cells with a normal quantity of granulations was observed in the pancreas of 5 non-diabetic dogs. Atrophy of the cortex was observed in the adrenals on the reticular and fasciculated layers.

A significant diminution in the frequency of apparition of diabetes and a lesser increase of the body weight were observed in castrated rats with partial pancreatectomy, treated with Prednisolone *(per os)* during 8 months at a dosis of 50 or 500 g/rat/day.

The histological study of the pancreas revealed hypertrophy and hyperplasia of the islets in non-diabetic treated rats. Atrophy of the cortex was observed in the adrenals.

The weight of the adrenal, spleen and thymus was less than in the controls.

Buenos Aires, May 3rd, 1956

Studies on Steroids. III) Corticosteroids in the peripheral blood. Isolation of hydrocortisone. C. P. DEL CAMPO DE HACHEN AND J. SOLÍS. *Departament of Endocrinology, Hospital Provincial, Alem 1450. Rosario, Argentina.*

Chromatographic procedures are used for isolation and identification of corticosteroids extracted from peripheral blood with butanol. THE, F and E are found as

the major compounds of healthy adult's blood. High levels of THE and minutes amounts of probable E are found in peripheral blood of two patients with Cushing's disease. Butanol's extracts give a zone at the starting line mixed with THE in Toluene-Propylene-Glycol system. This zone when is eluated and reapplied on paper Whatman Nº 1 Cloroform-Metanol 70 % system gives two zones one at the starting line and the other THE.

Influence of thyroid hormone on vascular reactivity to vasoactive substances in the rat. J. A. OSORIO. *Institute of Experimental Physiology, Faculty of Medicine. Porto Alegre, Brazil.*

Vascular reactivity to several drugs was studied in normal, hypothyroid and hyperthyroid rats.

Animals treated with thyroid powder showed a little less sensitivity to the following pressor drugs: epinephrine, norepinephrine and hypertensin when compared to normals. The hypothyroid group (I^{131}, propilthiouracil or surgery) showed a considerable diminution of sensitivity to these pressor agents.

On the contrary, hypothyroid rats treated with depressor substances (acetylcholine and tetraethylammonium chloride) were much more sensitive to these drugs than controls. This effect was more evident for acethylcholine than for TEAC, and might depend on a modification of the sensitivity of the cholinergic effectors of blood vessels.

Electrocardiographic records obtained during the fall of blood pressure caused by acetylcholine did not show any changes in heart rate. This might be explained as an anticipation of the vasodilator to the bradycardic effect of acetylcholine.

Estrogenic action of a tri-phenyl-ethylen derivate. G. POUMEAU-DELILLE. *Laboratory of Experimental Endocrinology, Viamonte 1819. Buenos Aires, Argentina.*

Bromo-α-diphenyl-β-para sthyl ethylen at a dosis of 1 mg daily, subcutaneously (a dosis ten times over the minimum active) provokes, in the castrated rat, incomplete periodical vaginal cycle; only proestrus is observed without cellular keratinization.

The same dosis of progesterone (1 mg, daily, subcutaneously) associated with Bromo-α-diphenyl-β-para ethyl phenyl ethylen decreases the response of the vaginal receptor without interruption of the artificial cycle.

A dissociation between the relative intensity of the progestative response of the uterine mucosa and the weakness of the decidual responses was observed after administration of both hormones at the same doses.

The estrogenic potency of 1 mg of this derivate of the triphenyl ethylene appears lower and less complete in the castrated rat (either adrenalectomized or not) than the estrogenic potency of an estradiol benzoate dosis of 0.05 to 0.1 μg.

Seasonal variation of the content of adrenaline and noradrenaline in the adrenal gland of the toad. E. C. RAPELA AND M. F. GORDON. *Institute of Biology Experimental Medicine, Costa Rica 4185. Buenos Aires, Argentina.*

The seasonal changes in the adrenaline and noradrenaline content of the adrenal gland of the male toad, *Bufo arenarum* Hensel have been studied. Colorimetric determinations have been made by the method of Euler and Hamberg (1949).

1) the content of adrenaline relative to total catechols (adrenaline-noradrenaline is high from September to March (Spring and Summer); decreases until May (Autumn) and recovers the higher values from July to August (Winter). The higher percentual value was observed in December, 50.4 % ± 2.0 and the minimum in May, 35.7 % ± 2.3.

2) the adrenaline content of the adrenal glands expressed as μg/toad was maximal in September (56.1 ± 6.3 g) decreasing until May when the minimum value was obtained (24.8 ± 2.3 μg).

3) the noradrenaline content expressed in the same way was also maximal in September (59.6 ± 7.5 μg) decreasing until March (39.7 ± 2.2 μg) and recovering the higher values from then on.

4) the lower adrenaline percentage relative to total catechols observed from April to August was due to an earlier recovering of the noradrenaline content and, particularly, to a longer and more marked decrease of adrenaline.

Buenos Aires, June 7th, 1956

Electrical stimulator for experimental and clinical Neurophysiology. M. TURNER AND M. RAPPAPORT. *Laboratorio de Electroneurofisiología Clínica, Sala XVII del Hospital de Niños y Servicio de Neurocirugía, Hospital Italiano. Buenos Aires.*

An electrical stimulator has been achieved with the following characteristics:

—Master oscilator covering one to one thousand pulses/sec. in different bands.

—Time delay, amplitude, pulses separation and pulses duration with independent controls, square waves ranging from 200 msec to 50 msec.

—Mixer and isolation stage coupled to constant impedance attenuator.

—Audio-amplifier stage, timing circuit to master oscilator and synchronising output for oscilloscope.

—Circuits and general diagram are provided.

Intrasplenic grafting of the ovary. E. FELS. *Instituto de Maternidad, Dirección Nacional de Asistencia Social. Buenos Aires, Argentina.*

When the intrasplenic grafting of the ovary in rats is carried out preserving its uterine connections, the blastomatous transformation, so common in the free graft, is only exceptionally produced. Ulterior separation of the uterine pedicle does not modify this situation. In hypothetical form, the difference existing between the intrasplenic graft of the free ovary and the pedicled ovary with or without ulterior section of the pedicle is explained.

Effect of Hypoglycemiant Sulphonamides in hypophysectomized, adrenalectomized or pancreatectomized animals. B. A. HOUSSAY AND J. C. PENHOS. *Instituto de Biología y Medicina Experimental, Costa Rica 4185. Buenos Aires, Argentina.*

The hypoglycemic action produced by sulphonamides BZ 55, 2254 RP and D 860 was observed in dogs, rats and toads.

The action was similar in the hypophysectomized and in the normal dogs at dosis of 200 mg/per kg of weight. However, the effect was more marked and the

mortality higher (6/10) in the hypophysectomized than in the controls (0/10). The curve of hypoglycemia was similar in the normal and in the hypophysectomized toads.

Adrenalectomized dogs and rats had a marked sensitivity to the hypoglycemic and toxic action of these sulphonamides, either injected or ingested.

Hypophysectomized animals showed a sensitivity to insulin more accentuated than the adrenalectomized ones.

In most cases, toxic symptoms were observed durin hypoglycemia, but in adrenalectomized-hypophysectomized dogs with partial pancreatectomy (removal of 82-85 % of the pancreatic mass) these toxic symptoms occurred when the blood sugar level was supernormal and they were not improved by injection of glucose. The toxicity of these sulphonamides on adrenalectomized dogs was not only due to hypoglycemia.

Hydrocortisone had a protective effect on hypoglycemic and, specially, on toxic symptoms observed in adrenalectomized rats.

The blood sugar level was not changed by sulphonamides in the absence of pancreas (pancreatectomized dogs or toads, pancreatectomized-hypophysectomized dogs).

Dogs deprived of adrenals, hypophysis and a partial part of the pancreas had a marked sensitivity to the toxic and hypoglycemic action of the sulphonamides. Their behaviour was similar to that observed in the adrenalectomized dogs.

Effect of the hypophysis on adrenal catechols in the toad. C. E. RAPELA AND M. F. GORDON. *Instituto de Biología y Medicina Experimental, Costa Rica 4185. Buenos Aires, Argentina.*

The adrenaline and noradrenaline content of the adrenal gland of the hypophysectomized male toad was studied. Colorimetric determination of tricloracetic acid extracts were made by the method of Euler and Hamberg (1949).

1. Hypophysectomy (ablation of the *pars distalis*) decreased the adrenaline percentage relative to total catechols (— 15.3 % — 2.3) from 2 to 7 days following the operation. The absolute adrenaline content decreased (— 21.9 — 2.7 g/sapo) but no changes were observed in the noradrenaline content.

2. Five days after hypophysectomy the adrenaline content was approximately half the amount of the controls.

3. Craneotomy did not produce any changes in the catechol content.

4. The subcutaneous injection of dry powder of toad hypophysis 1 mg/day, during 3 days, beginning one day following the operation, had no effect on the catechol content of hypophysectomized or hypophysectomized-castrated male toads.